

CP-1005

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Daniel Caput et al

Application Serial No. 09/077,817

Filing Date: September 14, 1998

Group Art Unit: 1635

Examiner: S. McGarry

For: IL-13 RECEPTOR

Assistant Commissioner for Patents  
Washington, D.C. 20231SUBMISSION OF CERTIFIED COPY AND SWORN  
TRANSLATION OF PRIORITY DOCUMENT

Under the provisions of 37 CFR 1.55(a) and pursuant to the claim for priority of French Application 95/14424 under 35 U.S.C. 119 made in the Declaration of above-identified U.S. Application Serial NO. 09/077,817, submitted herewith is a certified copy of said French application 95/14424 together with a sworn translation thereof.

Date

May 2, 2000

Address:  
Sanofi -Synthelabo Inc.  
9 Great Valley Parkway  
P.O. Box 3026  
Malvern, PA 19355  
Tel: (610) 889-6338  
Facsimile: (610) 889-8799

## CERTIFICATE UNDER 37 C.F.R. 1.8(a)

I hereby certify that this correspondence is being deposited on the date indicated below with the United States Postal Service as first class mail addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231

Name

Date

Joan Hylenki  
5 May 00

RECEIVED

MAY 15 2000

TECH CENTER 1600/2900

Respectfully submitted,

Paul E. Dupont  
Reg. No. 27,438

GREAT BRITAIN )  
ENGLAND )  
LONDON )

IN THE MATTER OF an Application  
for a US  
Patent in the name of

filed under No.



I, Susan POTTS BA ACIS,

do hereby certify:

THAT I am a Director of RWS Group plc, of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England and known as such to the undersigned Notary Public;

AND THAT, to the best of RWS Group plc knowledge and belief, the attached document, prepared by one of its translators competent in the art and conversant with the English and French languages, is a true and correct translation of the Specification

No. 95/14,424

filed by SANOFI

with their application for a Patent in France

on the 6th December 1995

for "IL-13 Receptor polypeptide"

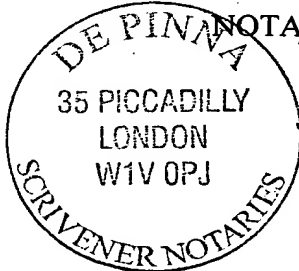
Signed by SUSAN POTTS )  
For and on behalf of RWS Group plc )  
this 25<sup>th</sup> day of April )  
2000 )

A handwritten signature in black ink, appearing to be "Susan Potts", written over a horizontal line.

SUSAN POTTS

I hereby certify the authenticity of the above signature of SUSAN POTTS whose identity I attest.

London, the 25<sup>th</sup> day of April 2000



A handwritten signature in black ink, written over a horizontal line.

NOTARY PUBLIC OF LONDON ENGLAND

D.N.L. FAWCETT

Scrivener Notary of London, England

**NIIP**

NATIONAL  
INSTITUTE OF  
INDUSTRIAL  
PROPERTY

---

**P A T E N T**

---

RECEIVED  
MAY 15 2000  
TECH CENTER 1600/2800

**UTILITY CERTIFICATE - CERTIFICATE OF ADDITION**

**OFFICIAL COPY**

The Director of the National Institute of Industrial Property certifies that the attached document is a true copy of an application for industrial property titleright filed at the Institute.

Drawn up in Paris, 21 NOV. 1996

On behalf of the Director of the National  
Institute of Industrial Property  
The Divisional Head

(signature)

Yves CAMPENON

NATIONAL  
INSTITUTE OF  
INDUSTRIAL  
PROPERTY

REGISTERED OFFICE  
26bis, rue de Saint Petersburg  
75800 PARIS Cédex 08  
Telephone: (1) 42 94 52 52  
Telefax: (1) 42 93 59 30

NATIONAL PUBLIC ESTABLISHMENT

CREATED BY LAW No. 51-444 OF 19 APRIL 1951

1 APPLICATION FOR THE GRANTING OF AN INDUSTRIAL PROPERTY TITLERIGHT*		2 COMPULSORY OPTIONS at the time of filing (except for utility certificate)	
		THE APPLICANT REQUESTS THE DIFFERED FORMULATION OF THE DOCUMENTATION REPORT	IF THE OPTION SELECTED IS NO AND IF THE APPLICANT IS A PHYSICAL PERSON HE REQUESTS THE GRADUATED PAYMENT OF THE TAX ON THE DOCUMENTATION REPORT
		<input type="checkbox"/> YES	<input type="checkbox"/> YES
		<input checked="" type="checkbox"/> NO	<input type="checkbox"/> NO
		NATURE NUMBER DATE OF INITIAL APPLICATION	
DATE OF SUBMISSION OF THE DOCUMENTS 06.DEC.1995	for c and d, state exactly : the nature, number and date of the initial application		
NATIONAL REGISTRATION No. 95/14.424	DATE OF FILING 06 DEC. 1995		
POSTAL CODE OF THE FILING PLACE 75	4 DATE OF THE GENERAL POWER OF ATTORNEY	5 REFERENCE OF THE CORRESPONDENT BFF 95/421	6 TELEPHONE No. OF THE CORRESPONDENT 48 74 92 22

7 TITLE OF THE INVENTION  
"IL-13 Receptor polypeptide"

8 APPLICANT: SURNAME AND CHRISTIAN NAMES (UNDERLINE THE SURNAME)  
OR NAME AND LEGAL CONSTITUTION

SIREN NO.

Company called : SANOFI

9 COMPLETE ADDRESS 32-34, rue Marbeuf 75008 PARIS		COUNTRY FR	
10 NATIONALITY French		TAXES PAID <input checked="" type="checkbox"/> ON FILING <input checked="" type="checkbox"/> ON DOCUMENTATION REPORT <input type="checkbox"/> ON CLAIM TO PRIORITY <input checked="" type="checkbox"/> ON CLAIM (from the 11th onwards)	
11 INVENTOR(S) THE APPLICANT IS THE SOLE INVENTOR? <input type="checkbox"/> YES If the answer is no see explanatory note <input checked="" type="checkbox"/> NO	12 IF THE APPLICANT IS A PHYSICAL PERSON NOT SUBJECT TO REVENUE COLLECTION, HE REQUESTS OR HAS REQUESTED REDUCTION OF THE TAXES <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO		
13 PRIORITY DECLARATION OR APPLICATION FOR THE BENEFIT OF THE FILING DATE OF A PRIOR APPLICATION	COUNTRY OF ORIGIN	FILING DATE	NUMBER
14 DIVISIONS PREVIOUS TO THE PRESENT APPLICATION No. No. No. No.			
15 SIGNATURE OF THE APPLICANT OR HIS REPRESENTATIVE NAME AND POSITION OF SIGNATORY REGISTRATION NO. CABINET LAVOIX C. POLUS No. 92.1200 (signature)	SIGNATURE OF THE RECEIVING OFFICIAL		SIGNATURE AFTER REGISTRATION OF THE APPLICATION AT THE N.I.I.P. (illegible signature)

\* Tick the relevant box

NIIP

NATIONAL INSTITUTE OF INDUSTRIAL PROPERTY

26bis, rue de Saint-Petersbourg 75800 Paris Cédex Tel: (1)42 94 52 52 -

Telecopier: (1)42 93 59 30

Patents Administrative Division

DESIGNATION OF THE INVENTOR

(if the applicant is not the  
inventor or the sole inventor)

National Registration No.

95/14,424

Title of the invention: IL-13 Receptor polypeptide

The undersigned

Company called : SANOFI  
32-34, rue Marbeuf 75008 PARIS FRANCE

designate(s) as inventor(s) (surname underlined, forenames,  
address)

Daniel CAPUT  
La Bousquière  
31290 AVIGNONET LAURAGAIS FRANCE

Pascual FERRARA  
Libouille Saint-Assiscle  
31290 AVIGNONET LAURAGAIS FRANCE

Patrick LAURENT  
Chemin Calmontais  
"Clochettes"  
31190 AUTERIVE FRANCE

Natalio VITA  
45bis, Chemin Al Cers  
31450 MONTGISCARD FRANCE

NOTE: In exceptional cases, the name of the inventor may be followed by that of the  
company to which he belongs (membership company) when the latter is other than the  
company which is the applicant or titleholder.

Date and signature(s) of the applicant(s) or of the  
representative

Paris, 6 December 1995

CABINET LAVOIX  
C. POLUS No. 92.1200  
(signature)

The present invention relates to a purified polypeptide having a receptor activity specific for interleukin-13 (IL-13), to its biologically active fragments and to the corresponding nucleic acid sequences and to its applications.

IL-13 is a recently identified (1,2) cytokine of 112 amino acids secreted by activated T lymphocytes, the B lymphocytes and the mastocytes after activation.

By virtue of its numerous biological properties shared with IL-4, IL-13 has been described as an IL-4-like cytokine. Its activities are indeed similar to those of IL-4 on the B cells (3-5), the monocytes (6-10) and other nonhaematopoietic cells (11-12). On the other hand, contrary to IL-4, it does not exert a specific effect on resting or activated T cells (13).

The various biological activities of IL-13 on the monocytes/macrophages, the B lymphocytes and certain haematopoietic precursors have been described in detail by A.J. Minty, as well as in review articles on IL-13 (see for example 14). Several data indicate, in addition, that this cytokine has a pleiotropic effect on other cell types. These nonhaematopoietic cells which are directly affected by IL-13 are endothelial and microglial cells, keratinocytes and kidney and colon carcinomas.

The anti-inflammatory and immunoregulatory activities of IL-13 may be useful, for example, in the treatment of autoimmune, tumour and viral pathologies.

An exploitation of these biological properties at the clinical level requires, however, a perfect knowledge of the signals and mechanisms via which these effects are exerted, so as to be able to control and modulate them in the relevant pathologies.

One of the stages in the analysis of the signal transmitted by a biological molecule within a cell consists in identifying its membrane receptor. The research studies carried out to this end on the IL-13 receptor have shown that IL-13 and IL-4 had a common receptor, or at the very least some of the components of a common receptor complex, as well as common signal

transduction elements (15-18). This receptor is present at the surface of various cell types, in a variable number according to the cell type considered. The comparative distribution of the IL-13 and IL-4 receptors has been indicated by A.J. Minty (14).

5 Kondo et al. (19) have described the structure of a receptor having a high affinity for IL-4. This receptor is a dimer, formed by the association of a glycoprotein of 140 kDa (IL-4R) and of the  $\gamma$  chain of the IL-2  
10 receptor ( $\gamma$ c). IL-4 can bind to the glycoprotein subunit of 140 kDa (IL-4R or gp 140) with a high affinity (Kd between 50 and 100 pM) (15). However, this affinity is increased by a factor of 2 to 3 when the  $\gamma$ c chain is associated with gp 140. This association is, in addition,  
15 necessary for the transmission of certain signals mediated by IL-4 (19,20).

Cross-competition experiments for binding either of IL-13 or of IL-4 have demonstrated that IL-4 can normally prevent the binding of IL-13, whereas IL-13 can  
20 generally only partially prevent the binding of IL-4 to its receptor (17,21) and does not attach to any of the two subunits of the IL-4 receptor or to the complex formed by their association. On the basis of these observations, the authors of the present invention have  
25 assumed that the receptor specific for IL-13 consisted of the receptor complex IL-4 associated with another IL-13 binding component.

Research studies carried out on an erythro-leukemic cell line capable of proliferating in response  
30 to IL-13 and IL-4 (TF-1 line) allowed them to show that these two cytokines produced similar intracellular events after attachment to their receptor (18). In parallel, cross-linking experiments allowed them to show that gp 140 could form heterodimers either with the  $\gamma$  chain, or  
35 with a new subunit, of a molecular weight of 55 to 70 kDa (17,21).

Given the importance, at the medical level, of the fine understanding of the phenomena of regulation of IL-4 and of IL-13, and in particular of the possibility

of being able to separate and control separately the effects produced by either of these two cytokines, the authors of the present invention were interested in the characterization of the subunit specifically binding  
5 IL-13 within the receptor complex common to IL-4 and IL-13.

These authors have now identified a human carcinoma cell line expressing the IL-13 specific receptor in a quantity greater than other known human  
10 renal carcinoma lines (21), and have carried out the cloning of the subunit responsible for the attachment of IL-13 to the IL-4/IL-13 receptor complex.

The present invention therefore relates to purified polypeptides specifically linking IL-13.

15 More particularly, the subject of the invention is a purified polypeptide whose amino acid sequence corresponds to that of a receptor specific for IL-13, or biologically active fragments of the latter.

The subject of the invention is also isolated DNA  
20 sequences encoding the said polypeptide or its biologically active fragments.

It relates, in addition, to the expression vectors containing at least one of the nucleotide sequences defined above, and the host cells transfected  
25 with these expression vectors under conditions allowing the replication and/or expression of one of the said nucleotide sequences.

The methods for producing the recombinant IL-13 receptor or its biologically active fragments by the  
30 transfected host cells are also part of the invention.

The invention also comprises pharmaceutical compositions comprising the receptor specific for IL-13 or biologically active fragments thereof for the regulation of the immunological and inflammatory mechanisms  
35 produced by IL-13. It relates, in addition, to a method for the identification of agents capable of modulating the activity of the receptor specific for IL-13, and the use of the IL-13 receptor or of fragments thereof for screening these agents as well as for the manufacture of



new products capable of modulating the activity of the IL-13 receptor.

5 The invention also comprises antibodies or derivatives of antibodies specific for the IL-13 receptor.

10 Finally, it relates to a method of therapeutic treatment for modulating the immunological reactions mediated by IL-13, comprising the administration, to a patient, of the receptor specific for IL-13 or for one of its biologically active fragments or of a compound capable of specifically modulating the activity of this receptor, in combination with a pharmaceutically acceptable vehicle.

15 In the description of the invention below, the following definitions are used:

- IL-13 receptor (IL-13R): a polypeptide comprising the amino acid sequence No. 2 or any biologically active fragment or derivative thereof;
- biologically active: capable of binding specifically to IL-13 and/or of participating in the transduction of the signal specifically produced by IL-13 at the level of the cell membrane, and/or capable of interacting with the receptor specific for IL-4 (IL-4R/gp 140) so as to form a complex capable of binding IL-4 and IL-13, and/or which is recognized by antibodies specific to the polypeptide of sequence SEQ ID No. 2, and/or capable of inducing antibodies which recognize the polypeptide of sequence SEQ ID No. 2;
- derivative: any polypeptide which is a variant of the polypeptide of sequence SEQ ID No. 2, or any molecule resulting from a modification of a genetic and/or chemical nature of the sequence SEQ ID No. 2, that is to say which is obtained by mutation, deletion, addition, substitution and/or chemical modification of one or of a limited number of amino acids, as well as any isoform sequence, that is to say a sequence which is identical to the sequence SEQ ID No. 2, to one of its fragments or modified sequences, containing one or more amino acids in the D enantiomer form, the said variant, modified or

isoform sequences having conserved at least one of the properties which make them biologically active.

The subject of the present invention is a purified polypeptide comprising an amino acid sequence  
5 chosen from:

a) the sequence SEQ ID No. 2,

b) any biologically active sequence derived from SEQ ID No. 2, according to the definition given above.

The manufacture of derivatives may have various  
10 objectives, including in particular that of increasing the affinity of the receptor for IL-13, that of enhancing its levels of production, of increasing its resistance to proteases, of modifying its biological activity or of conferring new pharmaceutical and/or biological pro-  
15 perties on it.

Among biologically active variants of the polypeptides as defined above, the fragments produced by alternate splicing of the transcripts (messenger RNAs) of the gene encoding one of the amino acid sequences  
20 described above are preferred.

In an advantageous variant, the 8 C-terminal amino acids of the polypeptide of sequence SEQ ID No. 2 are substituted by the following 6 amino acids:  
NH<sub>2</sub>-VRCVTL-COOH.

25 According to another advantageous aspect, the invention relates to a soluble form of IL-13R, comprising especially the extracellular domain of the polypeptide of sequence SEQ ID No. 2 stretching up to residue 343 and preferably up to residue 337.

30 The polypeptide which comprises the sequence SEQ ID No. 2 represents a specific embodiment of the invention. As will emerge in the examples, this polypeptide may be expressed at the surface of human cells so as to form a functional IL-13 receptor and  
35 combine with the IL-4 receptor so as to form, with the  $\gamma$  chain of the IL-2 receptor, the receptor complex common to IL-4 and IL-13.

The subject of the present invention is also an isolated nucleic acid sequence, chosen from:

- a) the sequence SEQ ID No. 1,
- b) the nucleic acid sequences capable of hybridizing to the sequence SEQ ID No. 1 and encoding a polypeptide having an IL-13 receptor activity,
- 5 c) the nucleic acid sequences derived from the sequences a) and b) because of the degeneracy of the genetic code.

More particularly, the subject of the invention is a sequence encoding the soluble part of IL-13R and any  
10 variant produced by alternate splicing of the transcripts of IL-13R, conserving at least one of the biological properties described.

A preferred embodiment is represented by a nucleic acid sequence comprising or consisting of the  
15 stretch of nucleotides stretching from nucleotide No. 1 up to nucleotide 1081, and preferably up to nucleotide 1063 on the sequence SEQ ID No. 1.

Another preferred embodiment is represented by a nucleic acid sequence comprising or consisting of the  
20 stretch of nucleotides stretching from nucleotide No. 1 up to nucleotide 1081, and preferably up to the nucleotide 1063 on the sequence SEQ ID No. 1.

Advantageously, the nucleic acid sequence according to the invention is a sequence encoding a protein  
25 corresponding to the mature form of the IL-13 receptor, this mature protein being the result of the release of the signal peptide.

The various nucleotide sequences of the invention may be of artificial origin or otherwise. They may be DNA  
30 or RNA sequences obtained by screening sequence libraries by means of probes produced on the basis of the sequence SEQ ID No. 1. Such libraries may be prepared by conventional molecular biology techniques known to persons skilled in the art.

35 The nucleotide sequences according to the invention may also be prepared by chemical synthesis or alternatively by a combination of methods including chemical or enzymatic modification of sequences obtained by screening of the libraries.

These nucleotide sequences allow the preparation of nucleotide probes capable of specifically hybridizing with a nucleic acid sequence, including a messenger RNA, encoding a polypeptide according to the invention or a  
5 biologically active fragment thereof. Such probes are also part of the invention. They may be used as an *IN VITRO* diagnostic tool for the detection, by hybridization experiments, of transcripts specific for the polypeptides of the invention in biological samples or for the detec-  
10 tion of aberrant syntheses or of genetic abnormalities resulting from a polymorphism, from mutations or from a poor splicing.

The probes of the invention comprise at least 10 nucleotides, and comprise at most the entire nucleotide  
15 sequence SEQ ID No. 1 or its complementary strand.

Among the shortest probes, that is to say of about 10 to 15 nucleotides, the appropriate hybridization conditions correspond to the temperature and ionic strength conditions customarily used by persons skilled  
20 in the art.

Preferably, the probes of the invention are labelled prior to their use. For that, several techniques are within the capability of persons skilled in the art (fluorescent, radioactive, chemiluminescent or enzymatic  
25 labelling, etc).

The *IN VITRO* diagnostic methods in which these nucleotide probes are used for the detection of aberrant syntheses or of genetic abnormalities, such as the loss of heterozygosity and genetic rearrangement, at the level  
30 of the nucleic sequences encoding an IL-13 receptor polypeptide or a biologically active fragment, are included in the present invention. Such a type of method comprises:

- bringing a nucleotide probe of the invention  
35 into contact with a biological sample under conditions allowing the formation of a hybridization complex between the said probe and the abovementioned nucleotide sequence, optionally after a preliminary step of amplification of the abovementioned nucleotide sequence;

- detection of the hybridization complex which may be formed;

- optionally, sequencing the nucleotide sequence forming the hybridization complex with the probe of the invention.

The cDNA probes of the invention may, in addition, be advantageously used for the detection of chromosomal abnormalities.

The nucleotide sequences according to the invention have, moreover, uses in the therapeutic field for the preparation of antisense sequences which may be used in gene therapy. The subject of the invention is thus antisense sequences capable of inhibiting, at least partially, the production of IL-13 receptor polypeptides as defined above. Such sequences advantageously consist of those which constitute the reading frame encoding IL-13R at the level of the transcript.

They may be more particularly used in the treatment of allergies and of inflammation.

The nucleotide sequences according to the invention may, moreover, be used for the production of recombinant polypeptides, as defined above, having an IL-13 receptor activity.

These polypeptides may be produced from the nucleotide sequences defined above, according to techniques for the production of recombinant products known to persons skilled in the art. In this case, the nucleotide sequence used is placed under the control of signals allowing its expression in a cellular host. The cellular host used may be chosen from prokaryotic systems, such as bacteria, or from eukaryotic systems, such as for example yeasts, insect cells, CHO cells (chinese hamster ovary cells) or any other system which is advantageously available commercially. A cellular host preferred for the expression of the polypeptides of the invention consists of the fibroblast line COS-7.

The signals controlling the expression of the polypeptides (promoters, activators, terminal sequences and the like) are chosen according to the cellular host

used. To this end, the nucleotide sequences according to the invention may be inserted into autonomously replicating vectors within the chosen host, or integrative vectors of the chosen host. Such vectors will be prepared  
5 according to the methods commonly used by persons skilled in the art, and the resulting clones may be introduced into an appropriate host by standard methods, such as for example electroporation.

The expression vectors containing at least one of  
10 the nucleotide sequences defined above are also part of the present invention.

In the case of the COS-7 cells, the transfection may be carried out using the vector pSE-1, as described in (17).

15 The invention relates, in addition, to the host cells transfected by these expression vectors. These cells may be obtained by the introduction, into host cells, of a nucleotide sequence inserted into a vector as defined above, followed by the culture of the said cells  
20 under conditions allowing the replication and/or expression of the transfected nucleotide sequence.

These cells may be used in a method for the production of a recombinant polypeptide of sequence SEQ ID No. 2 or a derivative, which method is itself included  
25 in the present invention and is characterized in that the transfected cells are cultured under conditions allowing the expression of a recombinant polypeptide of sequence SEQ ID No. 2 or a derivative, and in that the said recombinant polypeptide is recovered.

30 The purification processes used are known to persons skilled in the art. The recombinant polypeptide may be purified from cell lysates and extracts, from the culture supernatant, by methods used individually or in combination, such as fractionation, chromatographic  
35 methods, immunoaffinity techniques using specific mono-or polyclonal antibodies, etc.

The mono- or polyclonal antibodies capable of specifically recognizing the IL-13 receptor according to the definition given above are also part of the

invention. Polyclonal antibodies may be obtained from the serum of an animal immunized against the IL-13 receptor according to the usual procedures.

5 The monoclonal antibodies may be obtained according to the conventional hybridoma culture method described by Köhler and Milstein (Nature (1975) p.195).

Advantageous antibodies are antibodies directed against the extracellular domain of the IL-13 receptor.

10 The antibodies according to the invention are, for example, chimeric antibodies, humanized antibodies, Fab and F(ab')<sub>2</sub> fragments. They may also exist in the form of labelled antibodies or immunoconjugates. For example, they may be associated with a toxin, such as the diphtheria toxin or with a radioactive product. These  
15 immunotoxins may in this case constitute therapeutic agents which may be used for the treatment of certain pathologies involving an overexpression of the IL-13 receptor.

20 The antibodies of the invention, in particular the monoclonal antibodies, may also be used for the immunocytochemical analysis of the IL-13 receptors on specific tissue sections, for example by immunofluorescence, gold labelling, immunoperoxidase and the like.

25 They may thus be advantageously used in any situation or [sic] the expression of the IL-13 receptor needs to be observed (abnormal overexpression, monitoring of the regulation of membrane expression, etc).

30 The invention therefore also relates to a process for the *IN VITRO* diagnosis of pathologies correlated with an abnormal expression of the IL-13 receptor, in biological samples capable of containing the IL-13 receptor expressed at an abnormal level, characterized in that at least one antibody of the invention is brought into contact with the said biological sample, under  
35 conditions allowing the possible formation of specific immunological complexes between the IL-13 receptor and the said antibody(ies) and in that the specific immunological complexes which may be formed are detected.

The invention also relates to a kit for the *IN*

VITRO diagnosis of an abnormal expression of the IL-13 receptor in a biological sample and/or for measuring the level of expression of the IL-13 receptor in the said sample comprising:

- 5           - at least one antibody specific for the IL-13 receptor, optionally attached onto a support,
- means for revealing the formation of specific antigen/antibody complexes between the IL-13 receptor and the said antibody and/or means for quantifying these
- 10       complexes.

Another subject of the invention relates to a method for the identification and/or isolation of ligands specific for the IL-13 receptor or agents capable of modulating its activity, characterized in that a compound

15       or a mixture containing various compounds, optionally nonidentified, is brought into contact with cells expressing at their surface the IL-13 receptor, under conditions allowing interaction between the IL-13 receptor and the said compound, in the case where the

20       latter would have an affinity for the receptor, and in that the compounds bound to the IL-13 receptor, or those capable of modulating the biological activity thereof, are detected and/or isolated.

In a specific embodiment, this method of the

25       invention is adapted to the identification and/or isolation of agonists and of antagonists of IL-13 for its receptor.

The invention also comprises pharmaceutical compositions comprising, as active ingredient, a

30       polypeptide corresponding to the preceding definitions, preferably in a soluble form, combined with a pharmaceutically acceptable vehicle.

Such a polypeptide may indeed act in competition with IL-13R expressed at the cell surface, and thereby

35       constitute an antagonist specific for the binding of IL-13 to its receptor, which may be advantageously used for the synthesis of a medicinal product intended for modulating the reactions mediated by IL-13 in pathological situations.



Finally, the invention comprises a method for the therapeutic treatment of conditions linked to immunological reactions mediated by IL-13, comprising the administration to a patient of the receptor specific for IL-13 (or for one of its biologically active fragments), or of a compound capable of specifically modulating the biological activity thereof, in combination with a pharmaceutically acceptable vehicle.

Other characteristics and advantages of the invention will emerge in the rest of the description with the examples and the figures, of which the legends are represented below.

#### LEGEND TO THE FIGURES

- Figure 1: characterization of the IL-13R receptor present in Caki-1 cells.

a) Scatchard analysis (inset) of the saturation curve of IL-13 labelled with [<sup>125</sup>I];

b) binding of [<sup>125</sup>I][Phe43]-IL-13-GlyTyrGlyTyr in the presence of increasing concentrations of unlabelled IL-13 (•) and of IL-4 (o);

c) cross-linking experiments using radioactive IL-13 in the absence (lane a) and in the presence of a one hundred times excess of unlabelled IL-13 (lane b) or of IL-4 (lane c);

d) inhibition of the secretion of IL-6 induced by IL-13 and IL-4 in the presence of a monoclonal antibody specific for the IL-4R chain and the IL-4 antagonist Y124DIL-4.

- Figure 2: Nucleotide sequence of the cDNA of IL-13R, and comparison of the protein sequences of IL-5R and IL-13R.

a) nucleotide sequence of the cDNA of IL-13R. The amino acids corresponding to the deduced signal peptide of the nucleic sequence are indicated in italics and those corresponding to the transmembrane domain are indicated in bold characters. The potential N-glycosylation sites (Asn-X-Ser/Thr) are underlined;

b) alignment of the amino acids of the IL-13R and

IL-5R sequences. The protein sequences of human IL-13R and IL-5R are aligned as described above (22). The cysteine residues and the WSXWS motif which are characteristic of this family of receptors are boxed.

5       - Figure 3: patterns of expression of the IL-13R mRNA.

The RNA was prepared from the following cells: Caki-1 (lane a), A431 (lane b), TF-1 (lane c), U937 (lane d), Jurkat (lane e) and IM9 (lane f).

10       - Figure 4: characterization of the recombinant IL-13R.

The COS-7 cells are transfected with IL-13R cDNA and used for:

- 15       a) studies for the binding of radiolabelled IL-13 (inset) by Scatchard analysis of the saturation curve;
- b) cross-linking experiments using radiolabelled IL-13 in the absence (lane a) and in the presence of a one hundred times excess of unlabelled IL-13 (lane b);
- c-d) cotransfection experiments using cloned
- 20       IL-13R, IL-4R (gp140) and the  $\gamma$  chain followed by the binding of radiolabelled IL-13 (c) or of IL-4 (d). The black and white columns represent the specific binding of IL-13 and of IL-4 respectively.

25       - Figure 5: inhibition of the binding of IL-13 to IL-13R by the soluble form of the receptor (IL-13Rs) in transient expression.

30       The expression of IL-13Rs in the supernatant of the cells transfected with 2034 is tested by inhibition of the binding of IL-13 on cells transfected with IL-13R. The supernatants are tested in the crude state by diluting them one half in the iodinated ligand.

BT: total binding

NSB: nonspecific binding in the presence of an excess of unlabelled IL-13

35       2036 BT: total binding on cells transfected with 2036

2036 + sgt 2034: binding to cells transfected with 2036 in the presence of supernatant of cells transfected with 2034.

- Figure 6: inhibition of the binding of IL-13 to IL-13R by the soluble form of the receptor (IL-13R) on stable lines.

5      T2036-22: total binding on the clone IL-13R  
      (2036-22)  
          2034-4  
          2034-6  
          2034-19      4 clones IL-13Rs  
          2034-21  
10      1274-20:      CHO cells not expressing IL-13Rs.

## MATERIALS AND METHODS

### Binding and cross-linking experiments:

The binding and cross-linking experiments are carried out as described for [<sup>125</sup>I][Phe43]-IL-13-GlyTyrGlyTyr (17).

### Induction of the secretion of IL-6:

The Caki-1 cells (ATCC HTB46) are placed in 24-well plates at a density of 5x10<sup>4</sup> cells/well and after 3 days of culture confluent monolayers are washed three times with DMEM medium without foetal calf serum. The stimulation of the Caki-1 cells is carried out with 30 ng/ml of IL-4 or of IL-13 in the absence or in the presence of Y124DIL-4 or of an anti-gp140 monoclonal antibody. The quantity of IL-6 released into the culture medium after incubating for 24 hours is measured by an ELISA technique (Innotest, France).

### Isolation and analysis of IL-13R cDNA:

Total RNA was extracted from the Caki-1 cells as described above (23). The poly(A) RNA is isolated from the total RNAs with magnetic beads coated with oligo(dT)<sub>25</sub> (Dynal). A cDNA library containing 2x10<sup>5</sup> clones was constructed using the primer-adaptor procedure (24) and the vector pSE1 (25). The cloning strategy for the expression which was used has been previously described (17).

### Preparation of IL-13 cDNA:

The RNA samples are copied with reverse transcriptase and subjected to PCR using a sense primer corresponding to the sequence + 52 to + 71 and an antisense primer corresponding to + 489 to + 470 (the numbering is made on the basis of the cDNA sequence shown in Figure 2). The PCR-amplified products are hybridized with a probe complementary to sequences + 445 to + 461 of the cDNA. The size markers are indicated on the left of the figure.

### Characterization of the properties of IL-13R:

The COS-7 cells are transfected in Petri dishes as described above (17) and, 24 hours later, the cells are trypsinized and cultured in 24-well plates at a density of  $8 \times 10^4$  cells/well. After culturing for 48 hours at  $37^\circ\text{C}$ , the cells are used either for binding experiments (assays carried out in triplicate show a variation of less than 10%) or for cross-linking experiments with iodinated IL-13 as described. For the transfection, the COS-7 cells were transfected in 25-cm<sup>2</sup> plates using 0.6  $\mu\text{g}$  of various plasmids. After 24 hours, the cell monolayers are trypsinized and cultured in 12-well plates at the rate of  $8 \times 10^4$  cells/well. Three days later, the binding experiments are carried out with labelled IL-13 or IL-4. The results are representative of at least three experiments conducted independently.

### EXAMPLES

#### EXAMPLE 1:

Analysis of the expression of IL-13R at the surface of Caki-1 cells

It was recently discovered that human renal carcinoma cells expressed, in addition to the receptors shared by IL-4 and IL-13, a large excess of specific IL-13 receptors (21). On the basis of these results, a sample of human carcinoma cell lines was studied for the attachment of IL-13 as described above (17). A specific line, Caki-1 (ATCC HTB46), which expresses a particularly large number of binding sites for IL-13, was analysed in greater detail. The Scatchard curves obtained from saturation experiments show the presence of binding sites with a  $K_d$  of  $446 \pm 50$  pM and a capacity of  $7.2 \times 10^4$  receptors/cell (Figure 1a). In competition experiments, unlabelled IL-13 completely displaces labelled IL-13 in a dose-dependent manner, whereas IL-4 displaces with a high affinity about 10% of the labelled IL-13. Higher concentrations of IL-4 (greater than 100 nM) do not displace the remaining 90% of bound IL-13 (Figure 1b).

These results are in agreement with the existence of two sites, one shared by the two cytokines, the other specific for IL-13. The experiments on cross-linking by affinity for IL-13 show a complex of about 70 kDa, which coincides with the complex observed in similar cross-linking experiments with IL-13 in various cell types (17,21). Labelled IL-13 is completely displaced from the complex by IL-13 but not by IL-4, which is in agreement with the competition experiments (Figure 1c).

**EXAMPLE 2:**

Analysis of the secretion of IL-6 induced by IL-4 or IL-13.

The authors of the invention analysed the secretion induced by IL-4 or IL-13 on Caki-1 cells. The two cytokines induce the secretion of similar levels of IL-6, and the secretion is inhibited by antibodies specific for the  $\alpha$  chain of IL-4R and by the antagonist Y124DIL-4 (Figure 1d). This suggests that the receptors shared by the two cytokines in the Caki-1 cells are responsible for the induction of the secretion of IL-6. Similar results are observed when the phosphorylation of the protein complex IRS1/4PS (18) induced by IL-4 and IL-13 is analysed in the presence or in the absence of anti-IL-4R antibodies and of IL-4 antagonist.

These results, taken as a whole, suggest that the receptor complex IL-4/IL-13 expressed in the Caki cells is identical to that which was previously described and that the protein binding IL-13 (IL-13R) which is over-expressed is a component of the receptor responsible for the recognition of IL-13 in a functional complex which includes IL-4R.

These cells were therefore used as source of messenger RNA for the cloning of this IL-13 binding entity.

**EXAMPLE 3:**

Cloning of the IL-13 receptor

The strategy for the cloning and expression which

was used has been previously described (17). A cDNA library containing  $2 \times 10^5$  recombinant clones was constructed (24) using Caki-1 cells. The library was divided into batches of 1000 cDNAs in which the DNA of each batch, in plasmid form, was introduced into COS-7 cells (26). The binding of labelled IL-13 to the transfected COS-7 cells makes it possible to identify the batches of clones encoding an IL-13 receptor. The positive batches were distributed out and rescreened until a single clone capable of carrying out the synthesis of a cell surface protein capable of binding IL-13 was identified. Two independent IL-13R cDNAs were finally isolated. The complete nucleotide sequence of the IL-13R cDNA and the amino acid sequence deduced therefrom are shown in Figure 2a. The cDNA has a length of 1298 bases excluding the poly-A tail and has a short 3' untranslated region of 106 bases. A canonical AATAAA polyadenylation signal is in the expected place. The open reading frame between nucleotides 53 and 1192 defines a polypeptide of 380 amino acids. The sequence encodes a membrane protein with a potential signal peptide, a single transmembrane domain and a short intracytoplasmic tail.

Four potential N-glycosylation sites are located in the extracellular region. It is important to note that two consensus motifs considered as signatures of the type II family of cytokine receptors (27) are also present, the first being derived from an N-terminal disulphide bridge loop structure, the second being the WSXWS type motif located at the C-terminal end of the extracellular region. The very short cytoplasmic sequence might explain why it is only the receptor complex shared by IL-4 and by IL-13 in the Caki cells which transduces a signal in the cell.

Alignment studies demonstrate homologies with the human IL-5R  $\alpha$  chain (51% similarity and 27% identity, Figure 2b) and, to a lesser extent, with the prolactin receptor. It is interesting to note that the IL-5R complex consists of an  $\alpha$  chain which binds IL-5 but which needs another protein, the  $\beta$  chain shared with the IL-3

and GM-CSF receptors, to form a high-affinity receptor which is capable of transducing a signal (28).

**EXAMPLE 4:**

Detection of the IL-13R messenger RNAs in various  
5 cell lines

Surprisingly, in the Caki-1 cells, similar quantities of messenger RNAs for IL-13R and IL-4R are detected by Northern analyses although a large excess of IL-13R is expressed. This observation suggests that there  
10 is a greater translation of this mRNA compared with the IL-4R transcript and explains the lack of detection of the IL-13R mRNA in the cell lines expressing a small number of IL-13 binding sites. RT-PCR analyses (Figure 3) show that the transcript found in the Caki-1 cells is  
15 also present at lower levels in the keratinocytic line A431, the premyeloid cells TF-1, the premocytic cells U937 and the cell line B IM9. No transcript is detected in the Jurkat T cell line or in the pre-B NALM6 cell line. These results are in agreement with IL-13 binding  
20 studies on these same lines previously described by the authors of the present invention (17), and with the known biological targets of IL-13.

**EXAMPLE 5:**

Binding analyses carried out on COS-7 cells  
25 transfected with IL-13R cDNA

The COS-7 cells transfected with the isolated cDNA encoding IL-13R specifically bind labelled IL-13. The Scatchard analysis of the saturation curve shows a single component site with a Kd value of  $250 \pm 30$  pM and a  
30 maximum binding capacity of  $5.6 \times 10^5$  receptors/cell (Figure 4a).

The affinity of the recombinant receptor is in good agreement with the Kd value of 446 pM for IL-13R in the Caki-1 cells and for what has been described in  
35 several other cells (17). Consequently, in spite of a sequence homology with the  $\alpha$  chain of IL-5R, the cloned receptor behaves differently since it does not need a



second chain to reconstitute a high-affinity binding site.

It is interesting to note that the protein binding IL-15 recently described likewise has the characteristic of binding IL-15 with a high affinity, in the absence of the other two components of the IL-15R complex (29).

In competition experiments, IL-13 is capable of inhibiting the binding of labelled IL-13 to the cloned receptor, with an inhibitory constant ( $K_i$ ) of  $1.5 \pm 0.5$  nM, whereas IL-4 does not inhibit the binding. The pharmacology of the cloned receptor is therefore similar to that of the IL-13R present in Caki-1 cells. Cross-linking experiments show a radiolabelled band of 70 kDa. This band has the same mobility as that observed in the Caki cells as well as in other cells (17). This complex most probably corresponds to the 60-70 kDa band observed in addition to the IL-4R 140 kDa band in cross-linking experiments carried out with labelled IL-4. This could also suggest that a strong interaction exists between the two proteins in the functional receptor complex.

The authors of the present invention therefore checked if IL-13R and IL-4R interact in the cell membrane to reconstitute a receptor which allows cross-competition between the two cytokines. The results of a coexpression experiment are shown in Figure 4 c and d.

It appears clearly that the expression of the two receptors, either separately or simultaneously, results in a large number of receptors which specifically recognize either of the two cytokines. However, when they are expressed together, a small number of receptors (5 to 10%) is capable of recognizing the two cytokines. The cotransfection of the  $\gamma$ c chain with IL-4R and IL-13R does not bring about an increase in the number of shared binding sites. These results suggest that the IL-13R and IL-4R chains can interact with each other in the cell membrane to reconstitute a receptor for which IL-13 and IL-4 may be in competition. The low percentage of reconstituted receptors is an argument in favour of the

presence of another protein in limiting amounts in the COS cells which is necessary for the reconstitution of the receptor complex to which IL-13 and IL-4 bind competitively.

5           The results obtained in the transfection experiments with the  $\gamma$ c chain demonstrate that this protein is not the limiting factor which had previously been suggested (15). This conclusion is also supported by the absence of  $\gamma$ c messenger RNA in the Caki-1 cells (21).

10           Another possible reason which explains the low number of reconstituted receptors is the existence of an incorrect stoichiometry of the two proteins in the cell membrane. However, cotransfections using different relative quantities of IL-4R and IL-13R do not show a  
15   major difference in the number of reconstituted receptors. The possibility that another IL-13R with a greater capacity to interact with IL-4R exists cannot be excluded. It should be noted that the expression of  $\gamma$ c enhances the binding of IL-4 as previously described (19)  
20   but reduces the binding of IL-13, suggesting a complex interaction between the different chains.

#### EXAMPLE 6:

Study of the inhibition of the binding of IL-13 to its membrane receptor by a receptor in soluble form.

25           The results in transient expression (Figure 5) or on stable lines (Figure 6) are described.

          The two cDNA sequences encoding IL-13R and IL-13Rs are inserted into the vector p7055 in place of the IL-2 cDNA (30). The resulting plasmids are called  
30   2036 and 2034 respectively.

#### 1. Transient expression

          The CHO cells are inoculated into 12-well plates at  $3 \times 10^5$  cells/well and transfected the next day by the DEAE-Dextran method as for the COS cells, either with the  
35   plasmid 2036 or 2034, or with the empty plasmid pSEI [sic] as control.

          The cells are cultured for three days so as to

allow accumulation of IL-13Rs in the supernatant of the cells transfected with the plasmid 2034 and good expression of IL-13R at the membrane of the cells transfected with the plasmid 2036.

5           The supernatant of the cells transfected with IL-13Rs (2034) or the negative control (empty pSEI) [sic] is then collected and the cells transfected with IL-13R are used to study the inhibition of the binding of IL-13.

10           The binding of IL-13 to the surface of the CHO cells expressing IL-13R (2036) is measured in the presence or otherwise of these crude supernatants diluted one half with the radioligand or in the presence of an excess of nonradiolabelled IL-13 (NSB). The binding is carried out on whole cells in a final volume of 500  $\mu$ l  
15           with 300 pM of radioligand, in triplicate.

## 2.           Stable lines

Two stable transformed CHO lines are obtained by transfection with the coding sequences of the complete IL-13R (polypeptide of 380 residues) or of the IL-13R in  
20           soluble form (IL-13Rs, truncated polypeptide corresponding to residues 1 to 337 of IL-13R). These sequences are inserted into the vector p7055.

The CHO-DHFR<sup>-</sup> cells are transfected with the plasmids 2036 (IL-13R) and 2034 (IL613RS) and the  
25           recombinant clones selected as previously described (30).

One of the clones CHO-IL-13R (CHO 2036) obtained, having 2 to  $5 \times 10^5$  sites per cell, is inoculated into a 12-well plate at a density of  $10^5$  cells per well and the cells are used two days later for binding experiments in  
30           the presence or otherwise of IL-13Rs.

For that, the CHO-IL-13Rs (CHO 2034) clones are inoculated into 6 cm dishes, in triplicate, at  $5 \times 10^5$  cells per dish. After 3 days of accumulation in the culture medium, the medium (5 ml per dish) is collected  
35           for the IL-13 binding inhibition studies on IL-13R of the CHO 2036 clone. In the same manner, the supernatant of CHO cells not expressing the soluble IL-13R is collected.

The binding of IL-13 at the surface of the CHO

2036-22 clone is measured in the presence or otherwise of these crude supernatants diluted one half with the radioligand, or in the presence of an excess of nonradio-labelled IL-13 (NSB). The binding is carried out in triplicate, on whole cells, in a volume of 500  $\mu$ l with 300 pM of radioligand.

The histograms of Figures 5 and 6 represent the inhibition of the binding of IL-13 on IL-13R by IL-13Rs. Inhibition of the binding of IL-13 to its receptor can be observed on several clones.

The cloning of IL-13R described here makes it possible to improve the knowledge of the factors involved in the responses specifically induced by IL-13 compared with the responses induced by IL-4. It makes it possible, in addition, to have a tool for studying the regulation of the expression of the receptor under normal and pathological conditions where IL-13 plays a key role.

Moreover, the availability of cDNA makes it possible to facilitate the cloning of other proteins necessary for the reconstitution of an IL-4/IL-13 receptor complex and is also useful for the manufacture or the rational modelling of new medicinal products capable of being specific antagonists of the activities of IL-4 and of IL-13.

REFERENCES:

1. Minty, A., Chalon, P., Derocq, J.M., Dumont, X., Guillemot, J.C., Kaghad, M., Labit, C., Leplatots, P., Liauzun, P., Miloux, B., Minty, C., Casellas, P., Loison, G., Lupker, J., Shire, D., Ferrara, P. and Caput, D.  
5 (1993) Nature, 362, 248-250.
2. McKenzie, A.N., Culpepper, J.A., de Waal Malefyt, R., Briere, F., Punnonen, J., Aversa, G., Sato, A., Dang, W., Cocks, B.G., Menon, S., de Vries, J.E., Banchereau J. and  
10 Zurawski, G. (1993) Proc., Natl. Acad. Sci. U.S.A., 90, 3735-3739.
3. Defrance T, Carayon P, Billian G, Guillemot J-C, Minty A, Caput D, Ferrara P. J Exp. Med. 1994. 179: 135-143.
4. Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S,  
15 Zurawski G, de Waal Malefyt R, de Vries JE. Proc. Natl. Acad.Sci (USA) 1993; 90 : 3730-3734.
5. Fior R, Vita N, Raphael M, Minty A, Mailliot M-C, Crevon M-C, Caput D, Biberfeld P, Ferrara P, Galanaud P, Emilie D. Eur. Cytokine Network 1994. 5, 593-600.
- 20 6. Muzio M, Re F, Sironi M, Polentarutti N, Minty A, Caput D, Ferrara P, Mantovani A, Colatta F. Blood 1994. 83, 1738-1743.
7. de Waal Malefyt R, Figdor CG, Huijbens R, Mohan-Peterson S, Bennett B, Culpepper J, Dang W, Zurawski G,  
25 de Vries JE. J.Immunol. 1993 ; 151 : 6370-6381.
8. Doyle A, Herbein G, Montaner LJ, Minty AJ, Caput D, Ferrara P, Gordon S. Eur. J. Immunol. 1994. 24, 1441 - 1445.
9. Montaner LJ, Doyle AG, Collin M, Herbein G, Illei P,  
30 James W, Minty A, Caput D, Ferrara P, Gordon S. J Exp Med 1993. 178, 743-747.

10. Sozzani P, Cambon C, Vita N. Séguelas M-H. Caput D, Ferrara P and Pipy B. 1995. J. Biol. Chem. 270, 5084-5088.
- 5 11. Herbert JM, Savi P, Laplace MC, Lale A, Dol F, Dumas A, Labit C, Minty A. IL-4 and IL-13 exhibit comparable abilities to reduce pyrogen-induced expression of pro-coagulant activity in endothelial cells and monocytes. Febs Letters 1993 ; 328 : 268-270.
- 10 12. Derocq J-M, Segui M, Poinot-Chazel C, Minty A, Caput D, Ferrara P, Casellas P. Interleukin-13 stimulates interleukin-6 production by human keratinocytes. Similarity with interleukin-4. 18994. FEBS Lett. 343. 32-36.
- 15 13. Zurawski G, de Vries JE. Immunol. Today 1994. 15, 19-26.
14. Interleukin-13 for Cytokines in Health and Disease. Eds D.G. REMICK and J.S. FRIEDLAND, Marcel DECKER, N.Y. 1996.
- 20 15. Zurawski SM, Vega F, Huyghe B, Zurawski G. EMBO Journal 1993. 12, 2663-2670.
16. Aversa G, Punnonen J, Cocks BG, de Waal Malefyt R, Vega F, Zurawski SM, Zurawski G and de Vries JE. J. Exp. Med. 1993. 178, 2213-2218.
- 25 17. Vita N, Lefort S, Laurent P, Caput D, Ferrara P.J. Biol. Chem. 1995. 270, 3512-3517.
18. Lefort S, Vita N, Reeb R, Caput D and Ferrara P. FEBS Lett. 1995. 366, 122-126.
19. Kondo M. Takeshita T, Ishii N, Nakamura M, Watanabe S, Arai K, Sugamura K. Science 1993. 262, 1874-1883.

20. Russell SM, Keegan AD, Harada N, Nakamura Y, Noguchi M, Leland P, Friedman MC, Miyajima A. Puri PK, Paul WE, Leonard WJ., Science 1993. 262, 1880-1883.
21. Obiri N., Debinsky W., Leonard W.J., Puri R.K.  
5 J.Biol. Chem., 1995, 270, 8797-8804.
22. Devereux, J., Haeberli, P. & Smithies, O. Nucleic Acids Res. 12, 387-395 (1984).
23. Chomczynski, P. & Sacchi, N. Anal. Biochem. 162, 156-159 (1987).
- 10 24. Caput, D., Beutler, B., Hartog, K., Thayer, R., Brown-Schimer, S. and Cerami, A. Proc. Natl. Acad. Sci. USA 83, 1670-1674, 1986.
25. Minty, A., Chalon, P., Guillemot, J.C., Kaghad, M., Liauzun, P., Magazin, M., Miloux, B., Minty, C., Ramond, P., Vita, N., Lupker, J., Shire, D., Ferrara, P. and  
15 Caput, D. Eur. Cytokine Network. 4, 99-110, 1993.
26. Seed, B. and Arrufo, A. Proc. Natl. Acad. Sci. USA 84, 3365-3369, 1987.
27. Bazan, J.F. Proc. Natl. Acad. Sci. USA 87, 6934-6938,  
20 1990.
28. Honjo T. Current Opinion in Cell Biology 1, 201-203, 1991.
29. Giri, J.G., Kumaki, S., Ahdieh, M., Friend, D.J., Loomis A., Shanebeck, K., DuBose, R., Cosman, D., Park, L.S. and Anderson D.M. EMBO Journal 14, 3654-3663, 1993.  
25
30. Miloux, B. and Lupker, J.H. Rapid isolation of highly productive recombinant Chinese Hamster Ovary cell lines. Gene 14, 1994, 341-344.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: SANOFI
- 5 (B) STREET: 32 34 RUE MARBEUF
- (C) CITY: PARIS
- (E) COUNTRY: FRANCE
- (F) POSTAL CODE: 75374
- (G) TELEPHONE: 53774000
- 10 (H) TELEFAX: 53774133

(ii) TITLE OF INVENTION: Receptor IL-13

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- 15 (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release f1.0, Ver-
- sion f1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

- 20 (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1298 base pairs
  - (B) TYPE: nucleotide
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (F) TISSUE TYPE: Carcinoma



(G) CELL TYPE: renal  
(H) CELL LINE: Caki-1

(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 53..1192

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GCTGCTGTC GCGGGGAGA GAGCAATAT CAGGTTTTA AATCTGGAG AA ATG	56
Met	
1	
GCT TTC GTT TGC TTG GCT ATC GGA TGC TTA TAT ACC TTT CTG ATA AGC	103
Ala Phe Val Cys Leu Ala Ile Gly Cys Leu Tyr Thr Phe Leu Ile Ser	
5 10 15	
ACA ACA TTT GGC TGT ACT TCA TCT TCA GAG ACC GAG ATA AAA GTT AAC	151
Thr Thr Phe Gly Cys Thr Ser Ser Ser Asp Thr Glu Ile Lys Val Asn	
20 25 30	
GCT GCT CAG GAT TTT GAG ATA GTG GAT CCC GGA TAC TTA GGT TAT CTC	199
Pro Pro Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr Leu Gly Tyr Leu	
35 40 45	
TAT TTG CAA TGG CAA CCC CCA CTG TCT CTG GAT CAT TTT AAG GAA TGC	247
Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp His Phe Lys Glu Cys	
50 55 60 65	

ACA GTG GAA TAT GAA CTA AAA TAC CGA AAC ATT GGT AGT GAA ACA TGG Thr Val Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly Ser Glu Thr Trp 70 75 80	295
AAG ACC ATC ATT ACT AAG AAT CTA CAT TAC AAA GAT GCG TTT GAT CTT Lys Thr Ile Ile Thr Lys Asn Leu His Tyr Lys Asp Gly Phe Asp Leu 85 90 95	343
AAC AAG GGC ATT GAA GCG AAG ATA CAC AGC CTT TTA CCA TGG CAA TGC Asn Lys Gly Ile Glu Ala Lys Ile His Thr Leu Leu Pro Trp Gln Cys 100 105 110	391
ACA AAT GGA TCA GAA GTT CAA AGT TCG TCG GCA GAA ACT ACT TAT TGG Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr Trp 115 120 125	439
ATA TCA CCA GAA GGA ATT CCA GAA ACT AAA GTT CAG GAT ATG GAT TGC Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp Cys 130 135 140 145	487
GTA TAT TAC AAT TCG GAA TAT TTA CTC TGT TCT TCG AAA CCT GCG ATA Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly Ile 150 155 160	535
GGT GTA CTT CTT GAT ACC AAT TAC AAG TTG TTT TAC TCG TAT GAG GCG Gly Val Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu Gly 165 170 175	583
TTG GAT CAT GCA TTA CAG TGT GTT GAT TAC ATC AAG GCT GAT GGA CAA Leu Asp His Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly Gln 180 185 190	631
AAT ATA GGA TGC AGA TTT CCG TAT TTG GAG GCA TCA GAC TAT AAA GAT Asn Ile Gly Cys Arg Phe Pro Tyr Leu Glu Ala Ser Asp Tyr Lys Asp 195 200 205	679
TTG TAT ATT TGT GTT AAT GGA TCA TCA CAG AAC AAG CCT ATC AGA TCG Phe Tyr Ile Cys Val Asn Gly Ser Ser Glu Asn Lys Pro Ile Arg Ser 210 215 220 225	727
AGT TAT TTC ACT TTT CAG GTT CAA AAT ATA GTT AAA CCT TTG CCG CCA Ser Tyr Phe Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro Pro 230 235 240	775
GTC TAT CTT ACT TTT ACT CCG GAG AGT TCA TGT GAA ATT AAG CTG AAA Val Tyr Leu Thr Phe Thr Arg Glu Ser Ser Cys Glu Ile Lys Leu Lys 245 250 255	823
TGC AGC ATA CCT TTG GGA CCT ATT CCA GCA AGC TGT TTT GAT TAT GAA Trp Ser Ile Pro Leu Gly Pro Ile Pro Ala Arg Cys Phe Asp Tyr Glu 260 265 270	871
ATT GAG ATC AGA GAA GAT GAT ACT ACC TTG GTG ACT GCT ACA GTT GAA Ile Glu Ile Arg Glu Asp Asp Thr Thr Leu Val Thr Ala Thr Val Glu 275 280 285	919
AAT GAA ACA TAC AGC TTG AAA ACA ACA AAT GAA ACC CGA CAA TTA TGC Asn Glu Thr Tyr Thr Leu Lys Thr Thr Asn Glu Thr Arg Gln Leu Cys 290 295 300 305	957
TTT GTA GTA AGA AGC AAA GTG AAT ATT TAT TGC TCA GAT GAC GGA ATT Phe Val Val Arg Ser Lys Val Asn Ile Tyr Cys Ser Asp Asp Gly Ile 310 315 320	1015

TGC AGT GAG TCG AGT GAT AAA CAA TGC TCG GAA GGT GAA GAC CTA TCG	1063
Trp Ser Glu Trp Ser Asp Lys Gln Cys Trp Glu Gly Glu Asp Leu Ser	
325 330 335	
AAG AAA ACT TTG CTA CGT TTC TCG CTA CCA TTT GGT TTC ATC TTA ATA	1111
Lys Lys Thr Leu Leu Arg Phe Trp Leu Pro Phe Gly Phe Ile Leu Ile	
340 345 350	
TTA GTT ATA TTT GTA ACC GGT CTG CTT TTG GGT AAG CCA AAC ACC TAC	1159
Leu Val Ile Phe Val Thr Gly Leu Leu Leu Arg Lys Pro Asn Thr Tyr	
355 360 365	
CCA AAA ATG ATT CCA GAA TTT TTC TGT GAT ACA TGAAGACTTT CCATATCAAG	1212
Pro Lys Met Ile Pro Glu Phe Phe Cys Asp Thr	
370 375 380	
AGACATGGTA TTGACTCAAC AGTTTCGAGT CATGCCCAAA TTTTCAATAT GAGTCTCAAT	1272
AAACTGAATT TTTCTTGGCA ATGTTG	1298

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 380 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ala Phe Val Cys Leu Ala Ile Gly Cys Leu Tyr Thr Phe Leu Ile  
1 5 10 15

Ser Thr Thr Phe Gly Cys Thr Ser Ser Ser Asp Thr Glu Ile Lys Val  
20 25 30

Asn Pro Pro Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr Leu Gly Tyr  
35 40 45

Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp His Phe Lys Glu  
50 55 60

Cys Thr Val Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly Ser Glu Thr  
65 70 75 80

Trp Lys Thr Ile Ile Thr Lys Asn Leu His Tyr Lys Asp Gly Phe Asp  
85 90 95

Leu Asn Lys Gly Ile Glu Ala Lys Ile His Thr Leu Leu Pro Trp Gln  
100 105 110

Cys Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr  
115 120 125

Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp  
130 135 140

Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly  
145 150 155 160

Ile Gly Val Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu  
165 170 175

Gly Leu Asp His Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly  
180 185 190

Gln	Asn	Ile	Gly	Cys	Arg	Phe	Pro	Tyr	Leu	Glu	Ala	Ser	Asp	Tyr	Lys
195							200						205		
Asp	Phe	Tyr	Ile	Cys	Val	Asn	Gly	Ser	Ser	Glu	Asn	Lys	Pro	Ile	Arg
210						215					220				
Ser	Ser	Tyr	Phe	Thr	Phe	Gln	Leu	Gln	Asn	Ile	Val	Lys	Pro	Leu	Pro
225					230					235					240
Pro	Val	Tyr	Leu	Thr	Phe	Thr	Arg	Glu	Ser	Ser	Cys	Glu	Ile	Lys	Leu
			245					250						255	
Lys	Trp	Ser	Ile	Pro	Leu	Gly	Pro	Ile	Pro	Ala	Arg	Cys	Phe	Asp	Tyr
			260				265						270		
Glu	Ile	Glu	Ile	Arg	Glu	Asp	Asp	Thr	Thr	Leu	Val	Thr	Ala	Thr	Val
	275					280						285			
Glu	Asn	Glu	Thr	Tyr	Thr	Leu	Lys	Thr	Thr	Asn	Glu	Thr	Arg	Gln	Leu
	290					295					300				
Cys	Phe	Val	Val	Arg	Ser	Lys	Val	Asn	Ile	Tyr	Cys	Ser	Asp	Asp	Gly
305					310					315					320
Ile	Trp	Ser	Glu	Trp	Ser	Asp	Lys	Gln	Cys	Trp	Glu	Gly	Glu	Asp	Leu
			325						330					335	
Ser	Lys	Lys	Thr	Leu	Leu	Arg	Phe	Trp	Leu	Pro	Phe	Gly	Phe	Ile	Leu
			340					345					350		
Ile	Leu	Val	Ile	Phe	Val	Thr	Gly	Leu	Leu	Leu	Arg	Lys	Pro	Asn	Thr
	355						360						365		
Tyr	Pro	Lys	Met	Ile	Pro	Glu	Phe	Phe	Cys	Asp	Thr				
370						375					380				

CLAIMS

1. Purified polypeptide, comprising an amino acid sequence chosen from:
  - a) the sequence SEQ ID No. 2,
  - 5 b) any biologically active sequence derived from SEQ ID No. 2.
2. Polypeptide according to Claim 1, characterized in that it comprises the amino acid sequence SEQ ID No. 2.
- 10 3. Polypeptide according to Claim 1, characterized in that it is a variant form of the polypeptide of sequence SEQ ID No. 2 in which the 8 C-terminal residues are substituted by the following 6 residues: NH<sub>2</sub>-VRCVTL-COOH.
- 15 4. Polypeptide according to Claim 1, characterized in that it is a soluble form stretching up to residue 343 and preferably up to residue 337.
5. Isolated nucleic acid sequence encoding a polypeptide according to any one of the preceding claims.
- 20 6. Isolated nucleic acid sequence according to Claim 5, characterized in that it is chosen from:
  - a) the sequence SEQ ID No. 1,
  - b) the nucleic acid sequences capable of hybridizing to the sequence SEQ ID No. 1 and encoding a polypeptide
  - 25 having an IL-13 receptor activity,
  - c) the nucleic acid sequences derived from the sequences a) and b) because of the degeneracy of the genetic code.
7. Nucleic acid sequence according to Claim 6,
- 30 characterized in that it comprises or consists of the nucleotide linkage stretching from nucleotide No. 1 up to nucleotide 1081 and preferably up to nucleotide 1063 on the sequence SEQ ID No. 1.
8. Cloning and/or expression vector containing a
- 35 nucleic acid sequence according to any one of Claims 5 to 7.
9. Vector according to Claim 8, characterized in that it is the plasmid PSE-1.

10. Host cell transfected with a vector according to Claim 8 or 9.

11. Transfected host cell according to Claim 10, characterized in that it is a cell of the COS-7 line.

5 12. Nucleotide probe characterized in that it hybridizes specifically with any one of the sequences according to Claims 5 to 7, their complementary sequences or the corresponding messenger RNAs.

10 13. Probe according to Claim 12, characterized in that it comprises at least 10 nucleotides.

14. Probe according to Claim 12, characterized in that it comprises the whole of the sequence SEQ ID No. 1 or its complementary strand.

15 15. Antisense sequence capable of inhibiting, at least partially, the production of polypeptides according to any one of Claims 1 to 4, characterized in that it is chosen from the sequences constituting the reading frame encoding a polypeptide according to any one of Claims 1 to 4 at the level of the transcript.

20 16. Use of a sequence according to any one of Claims 5 to 7, for the preparation of diagnostic nucleotide probes or of antisense sequences which can be used in gene therapy.

25 17. Use of a probe according to any one of Claims 12 to 14, as *IN VITRO* diagnostic tool for the detection, by hybridization experiments, of the nucleic acid sequences encoding a polypeptide according to any one of Claims 1 to 4, in biological samples, or for revealing aberrant syntheses or genetic abnormalities such as the loss of  
30 heterozygosity or genetic rearrangement.

18. Use of a probe according to any one of Claims 12 to 14 for the detection of chromosomal abnormalities.

35 19. *IN VITRO* diagnostic method for the detection of aberrant syntheses or of genetic abnormalities at the level of the nucleic acid sequences encoding a polypeptide according to any one of Claims 1 to 4, characterized in that it comprises:

- bringing a nucleotide probe according to any one of Claims 12 to 14 into contact with a biological sample

under conditions allowing the formation of a hybridization complex between the said probe and the above-mentioned nucleotide sequence, optionally after a preliminary step of amplification of the abovementioned nucleotide sequence;

- detection of the hybridization complex which may be formed;

- optionally, sequencing the nucleotide sequence forming the hybridization complex with the probe of the invention.

20. Use of a nucleic acid sequence according to any one of Claims 5 to 7 for the production of a recombinant polypeptide according to any one of Claims 1 to 4.

21. Method for producing an IL-13 receptor recombinant polypeptide, characterized in that transfected cells according to Claim 10 or 11 are cultured under conditions allowing the expression of a recombinant polypeptide of sequence SEQ ID No. 2 or a derivative, and in that the said recombinant polypeptide is recovered.

22. Mono- or polyclonal antibodies, conjugated antibodies, or fragments thereof, characterized in that they are capable of specifically recognizing a polypeptide according to any one of Claims 1 to 4.

23. Use of the antibodies according to the preceding claim, for the purification or detection of a polypeptide according to any one of Claims 1 to 4 in a biological sample.

24. Process for the *IN VITRO* diagnosis of pathologies correlated with an abnormal expression of the IL-13 receptor in biological samples capable of containing the IL-13 receptor expressed at an abnormal level, characterized in that at least one antibody according to Claim 20 is brought into contact with the said biological sample, under conditions allowing the possible formation of specific immunological complexes between the IL-13 receptor and the said antibody(ies) and in that the specific immunological complexes which may be formed are detected.

25. Kit for the *IN VITRO* diagnosis of an abnormal



expression of the IL-13 receptor in a biological sample and/or for measuring the level of expression of the IL-13 receptor in the said sample comprising:

5       - at least one antibody specific for the IL-13 receptor according to Claim 22, optionally attached onto a support,

10       - means for revealing the formation of specific antigen/antibody complexes between the IL-13 receptor and the said antibody and/or means for quantifying these complexes.

26.       Method for the identification and/or isolation of polypeptides according to Claim 1 or agents capable of modulating their activity, characterized in that a compound or a mixture containing various compounds, 15 optionally nonidentified, is brought into contact with cells expressing at their surface a polypeptide according to Claim 1, under conditions allowing interaction between the polypeptide and the said compound, in the case where the latter would have an affinity for the polypeptide, 20 and in that the compounds bound to the polypeptide, or those capable of modulating the biological activity thereof, are detected and/or isolated.

27.       Ligand or modulator for a polypeptide as defined in Claims 1 to 4, capable of being obtained according to 25 the method of Claim 26.

28.       Pharmaceutical composition comprising, as active ingredient, a polypeptide according to any one of Claims 1 to 4.

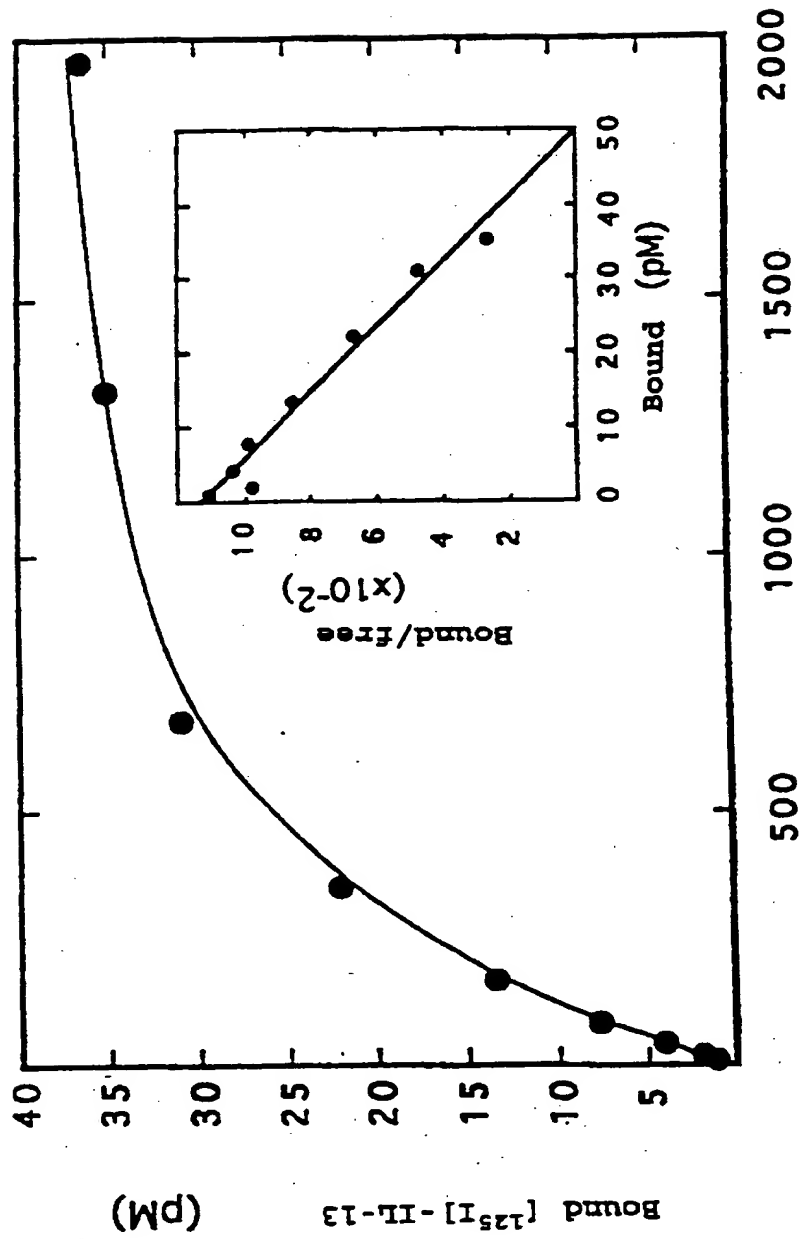
29.       Pharmaceutical composition according to the preceding claim, characterized in that it comprises a 30 polypeptide according to Claim 4.

30.       Use of a polypeptide according to any one of Claims 1 to 4, for screening agents capable of modulating the activity of IL-13R.

31.       Use of a polypeptide according to any one of Claims 1 to 4, for the manufacture of products capable of 35 modulating the activity of IL-13R.

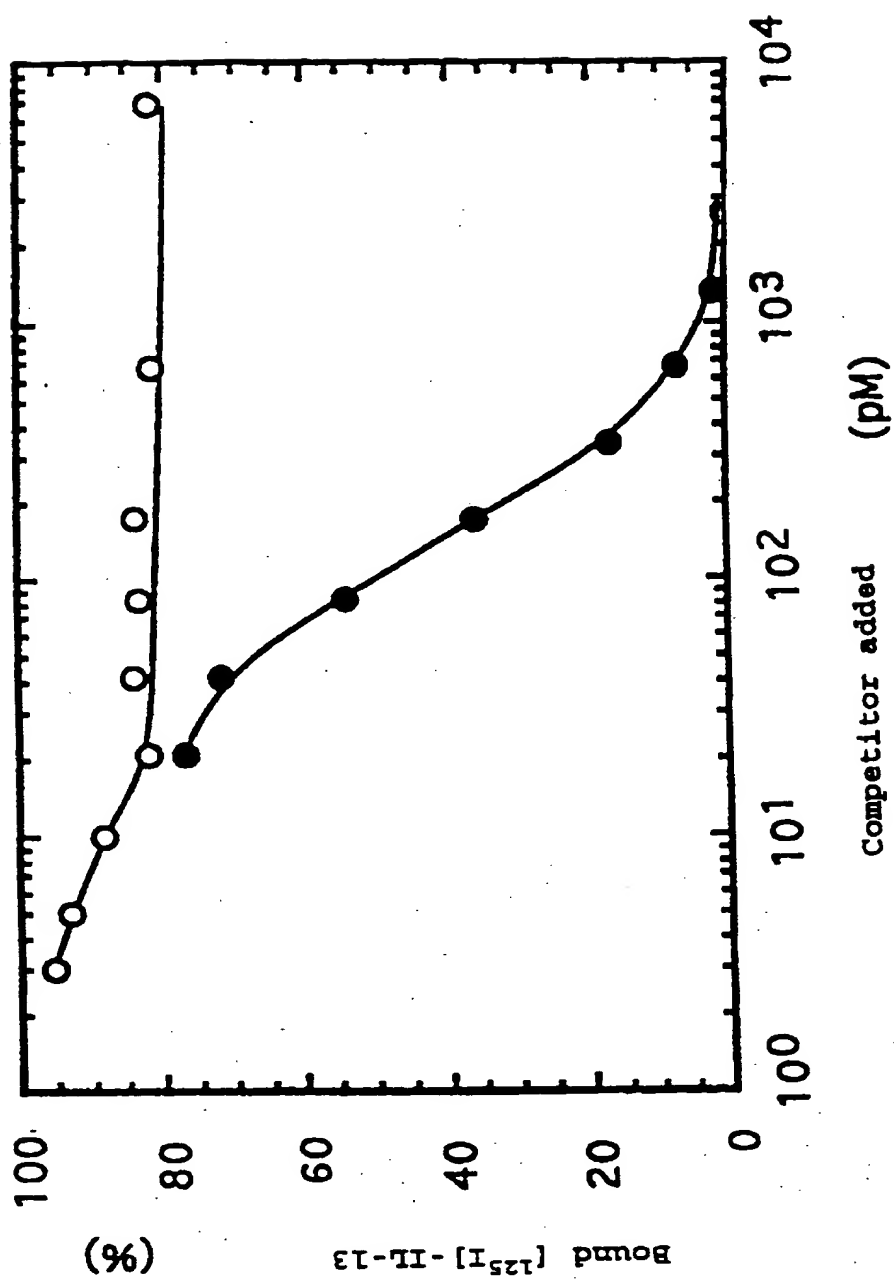
32.       Use of a polypeptide according to Claim 4, for the synthesis of a medicinal product with IL-13

antagonizing effect.



$[^{125}\text{I}]\text{-IL-13}$  (pM)

FIG. 1a

FIG.1b

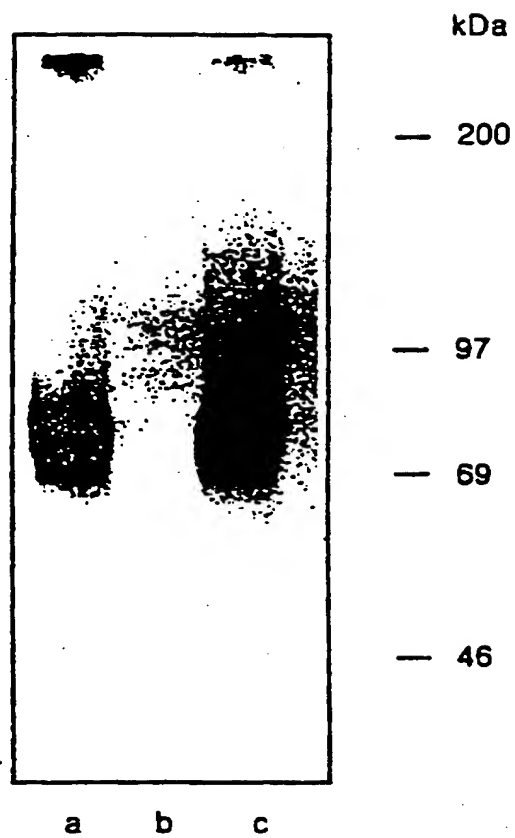


FIG. 1c

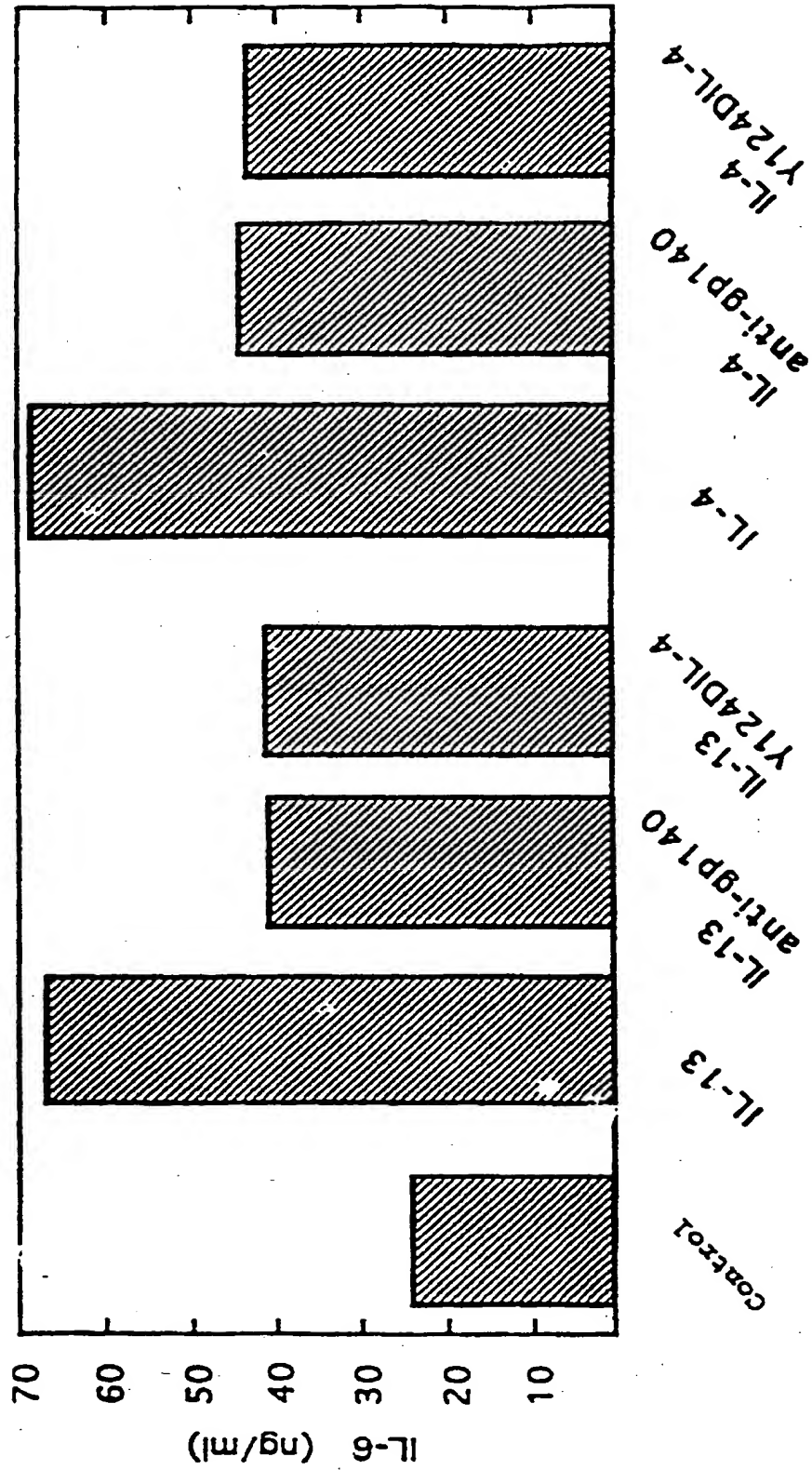


FIG.1d

1	GGTGCCTGTCGGCGGGGAGAGAGGCAATATCAAGGTTTTAAATCTCGGAGAAATGGCT	58
1	MetAla	2
59	TTCGTTTGCTTGGCTATCGGATGCTTATATACCTTTCTGATAAGCACAAACATTGGCTGT	118
3	PheValCysLeuAlaIleGlyCysLeuTyrThrPheLeuIleSerThrThrPheGlyCys	22
119	ACTTCATCTTCAGACACCGAGATAAAAGTTAACCTCCTCAGGATTTTGAGATAGTGGAT	178
23	ThrSerSerSerAspThrGluIleLysValAsnProProGlnAspPheGluIleValAsp	42
179	CCCGGATACTTAGGTTATCTCTATTTGCAATGGCAACCCCACTGTCTCTGGATCATTTT	238
43	ProGlyTyrLeuGlyTyrLeuTyrLeuGlnTrpGlnProProLeuSerLeuAspHisPhe	62
239	AAGGAATGCACAGTGAATATGAACTAAAAATACCGAAACATTGGTAGTGAACATGGAAG	298
63	LysGluCysThrValGluTyrGluLeuLysTyrArgAsnIleGlySerGluThrTrpLys	82
299	ACCATCATTACTAAGAATCTACATTACAAAGATGGGTTTGATCTTAACAAGGGCATTGAA	358
83	ThrIleIleThrLysAsnLeuHisTyrLysAspGlyPheAspLeuAsnLysGlyIleGlu	102
359	GCGAAGATACACACGCTTTTACCATGGCAATGCACAAATGGATCAGAAGTTCAAAGTTCC	418
103	AlaLysIleHisThrLeuLeuProTrpGlnCysThrAsnGlySerGluValGlnSerSer	122
419	TGGGCAGAACTACTTATTGGATATCACCACAAGGAATTCAGAACTAAAGTTCAGGAT	478
123	TrpAlaGluThrThrTyrTrpIleSerProGlnGlyIleProGluThrLysValGlnAsp	142
479	ATGGATTGCGTATATTACAATTGCAATATTTACTCTGTTCTTGAAACCTGGCATAGGT	538
143	MetAspCysValTyrTyrAsnTrpGlnTyrLeuLeuCysSerTrpLysProGlyIleGly	162
539	GTA CTCTTGATACCAATTACAATTGTTTTACTGGTATGAGGGCTTGGATCATGCATTA	598
163	ValLeuLeuAspThrAsnTyrAsnLeuPheTyrTrpTyrGluGlyLeuAspHisAlaLeu	182
599	CAGTGTGTTGATTACATCAAGGCTGATGGACAAAATATAGGATGCAGATTTCCCTATTTG	658
183	GlnCysValAspTyrIleLysAlaAspGlyGlnAsnIleGlyCysArgPheProTyrLeu	202
659	GAGGCATCAGACTATAAGATTTCTATATTTGTGTTAATGGATCATCAGAGAACAAGCCT	718
203	GluAlaSerAspTyrLysAspPheTyrIleCysValAsnGlySerSerGluAsnLysPro	222
719	ATCAGATCCAGTTATTTCACTTTTCACTTCAAATATAGTTAAACCTTTGCCGCCAGTC	778
223	IleArgSerSerTyrPheThrPheGlnLeuGlnAsnIleValLysProLeuProProVal	242
779	TATCTTACTTTTACTCGGAGAGTTTCATGTGAAATTAAGCTGAAATGGAGCATACCTTTG	838
243	TyrLeuThrPheThrArgGluSerSerCysGluIleLysLeuLysTrpSerIleProLeu	262
839	GGACCTATTCCAGCAAGGTGTTTTGATTATGAAATTGAGATCAGAGAAGATGATACTACC	898
263	GlyProIleProAlaArgCysPheAspTyrGluIleGluIleArgGluAspAspThrThr	282
899	TTGGTGA CTGCTACAGTTGAAAATGAAACATACACCTTGAAAACAACAAATGAAACCCGA	958
283	LeuValThrAlaThrValGluAsnGluThrTyrThrLeuLysThrThrAsnGluThrArg	302
959	CAATTATGCTTTGTAGTAAGAAGCAAAGTGAATATTTATTGCTCAGATGACGGAATTTGG	1018
303	GlnLeuCysPheValValArgSerLysValAsnIleTyrCysSerAspAspGlyIleTrp	322
1019	AGTGAGTGGAGTGATAACAATGCTGGGAAGGTGAAGACCTATCGAAGAAAACCTTGCTA	1078
323	SerGluTrpSerAspLysGlnCysTrpGluGlyGluAspLeuSerLysLysThrLeuLeu	342
1079	CGTTTCTGGCTACCATTTGGTTTCATCTTAATATTAGTTATATTTGTAACCGGTCTGCTT	1138
343	ArgPheTrpLeuProPheGlyPheIleLeuIleLeuValIlePheValThrGlyLeuLeu	362
1139	TTGCGTAAGCCAAACACCTACCCAAAAATGATTCCAGAATTTTCTGTGATACATGAAGA	1198
363	LeuArgLysProAsnThrTyrProLysMetIleProGluPhePheCysAspThr	381
1199	CTTTCATATCAAGAGACATGGTATTGACTCAACAGTTTCCAGTCATGGCCAAATGTTCA	1258
1259	ATATGAGTCTCAATAAACTGAATTTTTCTTGCGAATGTTG 1298	

FIG. 2a

IL13R	MAFVCLAIGCLYTFLISTTFGCTSSSDTEIKVNPPQDFEIVDPGYLGYLEY	50
IL5R	..MIIVAHVLLILLGATEILQADLLPDEKISLLPPVNFTIKVTG.LAQVL	47
IL13R	LQWQPPLSLDHFKECTVEYELKYRNIGSETWKTIIITKNLHYKDGFDLNKG	100
IL5R	LQWKPNPDQEQ.RNVNLEYQVKINAPKEDDYETRITES...KCVTILHKG	93
IL13R	IEAKIHTLLPWQCTNGSEVQSSWAETTYWISPOGIPETKVQDMDQV....	146
IL5R	FSASVRTILQ...NDHSLASSWASAE.LHAPPGSPGTSIVNLTTITNTT	139
IL13R	..YYNWQ.....YLCSWKPGIGVLLDTNYNLFYWYEGLDHALQVVDYIK	189
IL5R	EDNYSRLRSYQVSLHCTWLVGTDAPEDTQYFLYYRYGSWTE..EQEYSK	187
IL13R	AD.GQNIQCRFP..YLEASDYKDFYICVNGSSSENKPIRSSYFTFQLNIV	236
IL5R	DTLGRNIAQWFPRTFILSKGRDWLSVLVNGSSKHSAIRPFDQLFALHAID	237
IL13R	KPLPPVYLTFRESSCEIKLKWSIPLGPIPARCFDYEIEIREDDTTLVTA	286
IL5R	QINPPLNVTAEIEGT.RLSIQWEKPVSAPFIHCFDYEYVKIHNRNGYLQI	286
IL13R	TVENETYTLKTTNETRQLCFVVRSKVNIYCSDDGIIWSEWSDKQCWEGEDL	336
IL5R	EKLMTNAFISIIDDLSKYDVQVRAAVSSMCREAGLIWSEWSQ.PIYVGND	335
IL13R	SKKTLLRFWLPFGFILILVIFVTGILLRKPNTYPMIP.....EF	376
IL5R	HKPLREWFVIVIMATICFILLILSLICKICHLWIKLFPPIPAPKSNIKDL	385
IL13R	FCDT.....	380
IL5R	FVTNNYEKAGSSETEIEVICYIEKPGVETLEDSVF	420

FIG. 2b



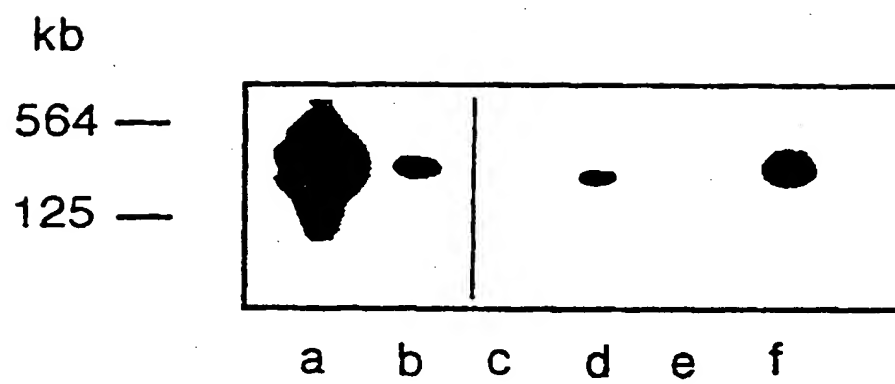


FIG.3

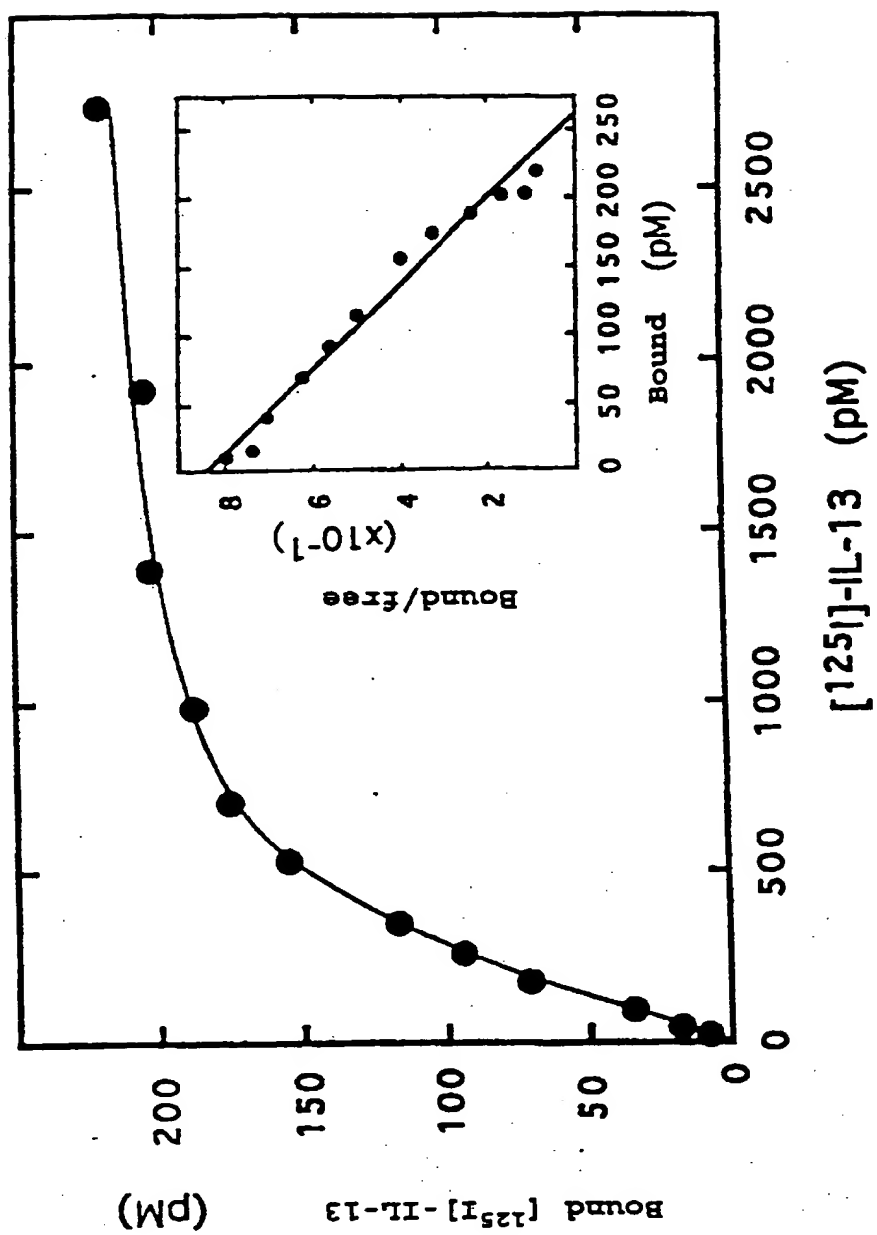


FIG. 4a

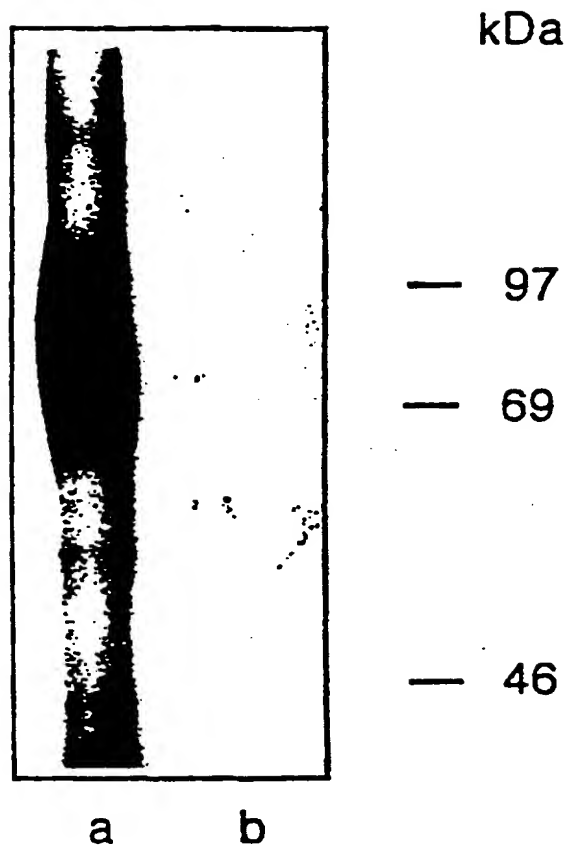


FIG.4b

FIG. 4C

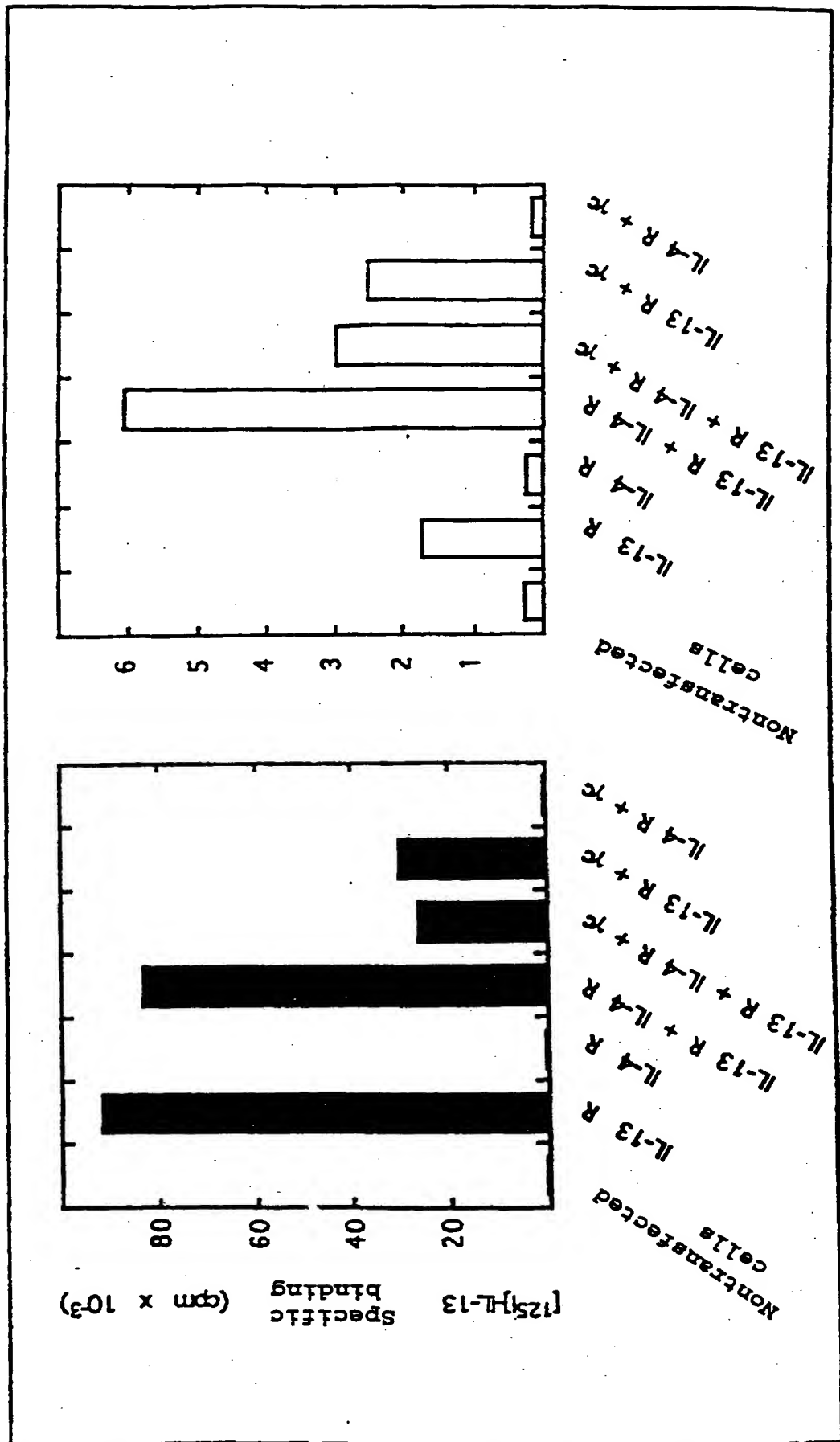
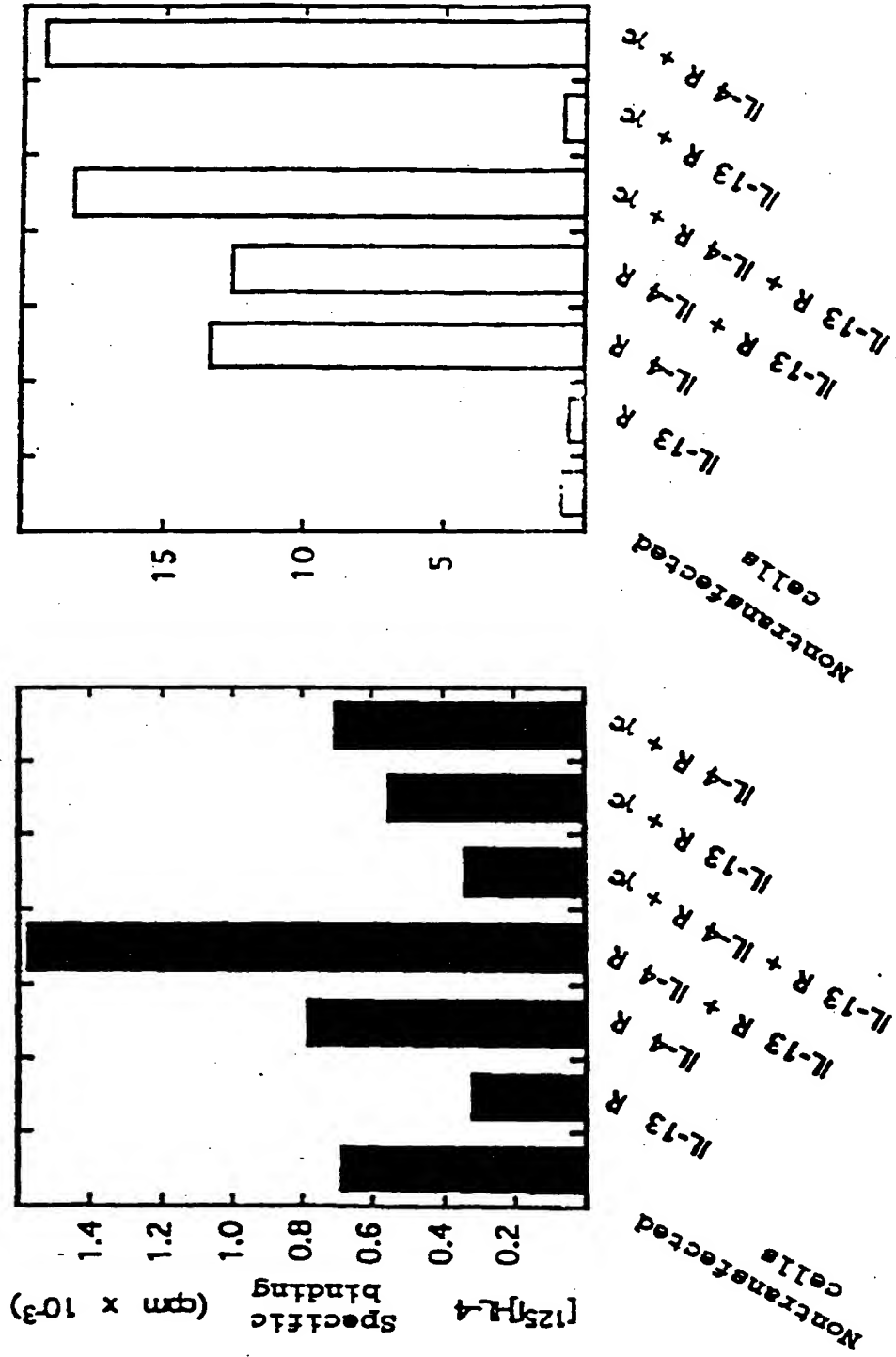


FIG. 4d



12/12

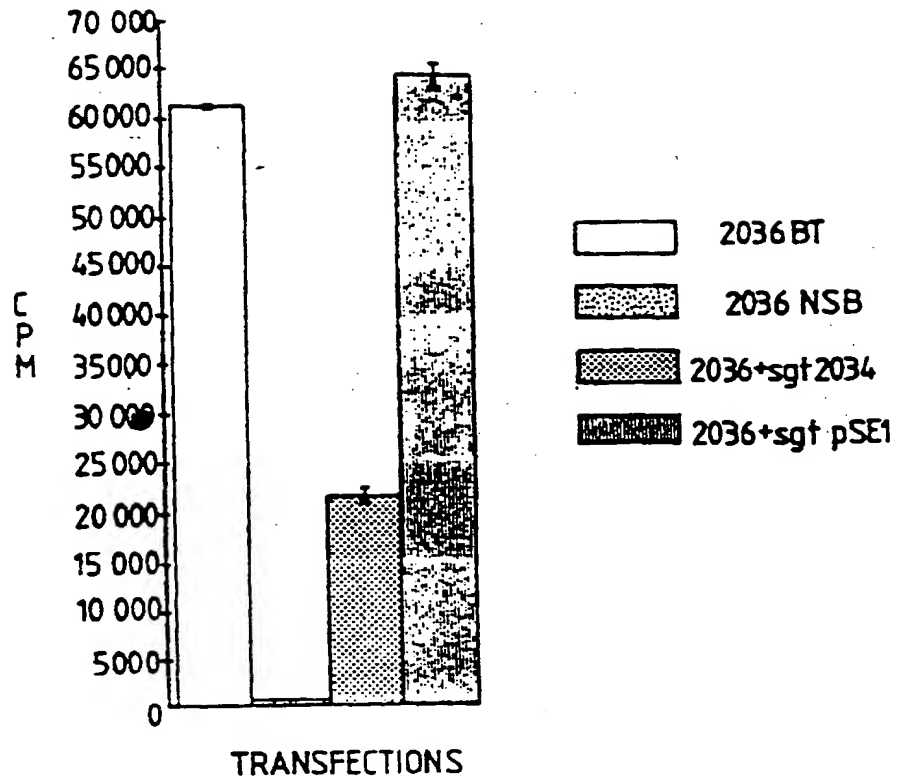


FIG. 5

FIG. 6

